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(54) Milk protein partial hydrolysate and process for preparation thereof

Teilhydrolysat aus Milcheiweiss und Verfahren zu dessen Herstellung

Hydrolysat partiel de protéines de lait, et procédé de préparation

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- **JOURNAL OF FOOD SCIENCE, vol.57, no.5, 1992, CHICAGO pages 1223 - 1229 M.I. MAHMOUD ET AL 'ENZYMATIC HYDROLYSIS OF CASEIN: EFFECT OF DEGREE OF HYDROLYSIS ON ANTIGENICITY AND PHYSICAL PROPERTIES'**

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Description

[0001] The present invention relates to a milk protein partial hydrolysate, a process for the enzymatic preparation of a milk protein partial hydrolysate and infant formula products of reduced antigenicity containing said hydrolysate.

[0002] A large number of infant formulas are based on proteins from cow's milk. Infants who are truly allergic to milk protein require infant formulas wherein the proteins are extensively hydrolyzed to contain a minimum of residual molecular structures. For non-allergic infants, an infant formula with reduced antigenicity has prophylactic benefits in that it can delay or prevent sensitization which could otherwise lead to clinical symptoms of allergy. The allergenic potential of cow milk-protein based formulas can be reduced by protein hydrolysis.

[0003] To ideally meet the composition of human milk, the cow milk protein in infant formulas should contain both whey protein and casein in an appropriate ratio. While a number of products based on intact milk protein meet a desirable whey protein to casein ratio, almost all of the commercially available partially hydrolyzed formulas are based on 100% whey protein.

[0004] Processes for preparation of partial hydrolysates described in the literature generally involve multi-step hydrolysis and physical separations after the hydrolysis to eliminate enzymes and/or residual proteins. Most processes also involve constant pH control during hydrolysis. Also, unless another process step is introduced, the resulting hydrolysate will usually have a high level of salts which can pose formulation problems in the infant formula, the level of minerals of which are usually regulated at a certain level.

[0005] U.S. Patent 5,039,532 describes two steps of enzymatic hydrolysis to attain a hydrolysate of desired characteristics. It also describes preparation of an infant formula which is ultra-high temperature (UHT) sterilized.Gmoshinsky et al. describe in Vopr. Pitan. 3, 21-27 (1991) milk protein-derived preparations with decreased antigenicity. U.S. Patents 4,293,571 and 4,981,704 both describe partial hydrolysates prepared using pancreatic enzymes which involve post-hydrolysis membrane processes to separate out the residual proteins and enzymes.

[0006] A common characteristic of protein hydrolysates, particularly hydrolysates containing casein, is bitter flavor development putatively due to liberation of peptides with hydrophobic end groups. Moreover, the emulsifying property of proteins generally is also decreased as the degree of hydrolysis increases. Japanese Patent 1160458 describes a milk protein hydrolysate (5-20% hydrolyzed) which is surface active and shows emulsifying activity in foods such as ice cream and whipping cream. However, in products such as liquid infant formulas which undergo sterilization processes, further denaturation of the protein hydrolysate renders it less functional in an emulsion. This is manifested in the product as a separation into a serum and cream layer. The high temperature-short time conditions of UHT sterilization is commonly preferred to conventional retort sterilization to prevent the adverse exposure to heat.

[0007] Thus, it would be highly desirable to have a partial protein hydrolysate which has reduced antigenicity, has a whey protein to casein ratio which provides a protein composition similar to human milk, has improved taste, and/or has improved emulsifying activity. The present invention is directed to a partial hydrolysate of a protein mixture wherein said protein mixture comprises whey protein and casein and wherein the hydrolysate has a degree of hydrolysis between 4 and 10% and to an infant formula containing said partial hydrolysate.

[0008] The present invention is also directed to a process for preparing a partial hydrolysate of a protein mixture comprising contacting a mixture of whey protein and casein with an enzyme mixture comprising at least 1800 USP Trypsin Units/mg and at least 350 USP Chymotrypsin Units/mg in an aqueous suspension under conditions to result in a degree of hydrolysis between 4 and 10%, wherein the ratio in USP units of Trypsin to Chymotrypsin is 1.3 to 18.

[0009] The present invention provides a partial hydrolysate of a mixture of whey proteins and casein which is appropriate to the production of nutritional products of reduced antigenicity, as well as a process for its preparation. The partial hydrolysate of the invention also is closer to the whey/casein protein ratio of human milk as compared to prior art partial protein hydrolysates. Additionally, the partial hydrolysate of the invention preferably has improved taste and improved emulsifying activity.

[0010] The process according to the invention may be carried out by using starting materials consisting of mixtures of whey protein and casein preferably in ratios similar to that found in human milk. Preferably the protein mixture comprises 60 to 80 % whey protein and 40 to 20 % casein, more preferably is 40 to 60 % whey protein and 60 to 40 % casein. Percentages of casein and whey protein are expressed on a weight basis. The whey proteins may be sourced from a whey obtained from cheese making, particularly a sweet whey such as that resulting from the coagulation of casein by rennet. The whey proteins may also be used in the form of concentrates in the range of 35-80 % protein as obtained by ultrafiltration (UF whey). This whey material, optionally, may also be demineralized by ion exchange and/or electrodialysis (ED whey). The casein source can either be acid casein or non-fat milk solids (NFDM). The whey protein and casein can be used either in the form of liquid concentrates or powders. For the process of this invention, the proteins are diluted or reconstituted to solutions containing 10 to 60 g protein per liter, preferably 40 to 60 g protein per liter.

[0011] For the process of the present invention, a mixture of trypsin and chymotrypsin, the enzymatic specificities of which are complementary, is used. The enzyme mixture preferably is free of other contaminating proteases such as

carboxypeptidase A and B or leucine aminopeptidase. The trypsin in the mixture has equal to or greater than 800 USP units/mg, preferably equal to or greater than 1000 USP units/mg, and more preferably equal to or greater than 1800 USP units/mg. One USP Trypsin Unit is the activity causing a change in absorbance of 0.003 per minute under the conditions of 5 minutes assay time, pH 7.6, 25 ± 0.1°C and N-benzoyl-L-arginine ethyl ester hydrochloride (BAEE) as substrate.

[0012] The chymotrypsin in the mixture has equal to or greater than 150 USP units/mg, preferably equal to or greater than 200 USP units/mg more preferably equal to or greater than 350 USP units/mg. One USP Chymotrypsin Unit is the activity causing a change in absorbance of 0.0075 per minute under the conditions shown under the conditions of 5 minutes assay time, pH 7.6, 25 0.1°C with N-acetyl-L-tyrosine ethyl ester (ATEE) as substrate.

[0013] Commercial sources of enzymes suitable for use in the present invention include Novo Nordisk Bioindustrials, Inc., Danbury, CT, U.S.A., (particularly PEM 2500S), Enzyme Development Corp., New York, NY, U.S.A., Intergen Company, Purchase, NY, U.S.A., and Scientific Protein labs., Waunakee, WI, U.S.A.

[0014] To obtain a hydrolysate of desirable properties, it is typically necessary that the mixture of trypsin and chymotrypsin have a trypsin to chymotrypsin ratio of 1.3 to 18 in the USP units described above, preferably 1.3 to 10, and more preferably 4 to 6. For the purpose of the invention, the enzyme mixture typically is used at levels of 0.4% to 1.2% by weight of the total protein being hydrolyzed, preferably 0.6% to 0.8% by weight.

[0015] An optional preliminary step prior to hydrolysis is preheating of the protein solution to insure denaturation of whey protein fractions e.g., serum albumin (BSA) and immunoglobulins (particularly IgG). This step usually results in a diminished residual antigenicity when assessed immunochemically (as described hereinafter). The pretreatment step is typically performed by heating to 75°C to 85°C for 10 minutes to 30 minutes. The hydrolysis itself is typically conducted at temperatures of 30°C to 50°C for 2 to 6 hours, the lower temperature limit corresponding to the upper time limit and vice versa. Maintenance of pH typically is not required during hydrolysis, since the hydrolysis usually proceeds at pH 6.5-6.8 without additional pH control. The pH should be kept within the range of 6.5 to 8.0, with or without pH control.

[0016] Irrespective of the conditions of the hydrolysis, the hydrolysate preferably is subjected to an additional step of enzyme inactivation. This enzyme inactivation can be a heat treatment which comprises heating to a temperature of 85°C for 10 minutes. Alternatively, the enzyme may be inactivated by sterilization at ultra-high temperature (e.g., 130°C for 45 seconds) after which the product can be stored in a liquid state. The hydrolysate may also be concentrated by evaporation or dried by spray drying.

[0017] To monitor the degree of hydrolysis, the United States Pharmacopeia (USP) formol titration method is used wherein the increase in free amino groups during the hydrolysis of peptide bonds can be estimated by titration with sodium hydroxide. The degree of hydrolysis is between 4% and 10%, preferably between 5% and 7%. It is an advantage of the present invention that the degree of hydrolysis is lower than achieved in certain prior art processes; yet a significant reduction in antigenicity is still obtained.

[0018] Size exclusion chromatography (SEC) is used for determination of hydrolysate peptide molecular weight distribution. The peptides in the hydrolysate are separated according to molecular size in a TSK G-2000 SWXL column maintained at 37°C and eluted at 0.7 ml/minute with TFA and acetonitrile in KCl. Absorption at 214 nm vs. retention time is generated with a UV detector and compared with those of standard proteins and peptides of known molecular weight. The partial hydrolysate of the invention preferably has an average molecular weight of 2,000, a maximum molecular weight of 19,000 and is comprised of peptides with the following distribution, as a function of their molar mass:

Molar Mass (g per mole)	% Molecular Weight Distribution
MM > 5000	8.2
5000 > MM >3000	14.5
3000 > MM > 2000	15.8
2000 > MM > 1000	26.2
1000 > MM > 500	17.8
MM < 500	17.5

[0019] The hydrolysate of the invention is preferably devoid of detectable intact milk protein. The absence of intact milk protein in the hydrolysate is demonstrated in sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE is performed with a 4 to 20% Tris-glycine gradient gel. Fifteen micrograms of hydrolyzed protein is treated with SDS, reduced with 2-mercaptoethanol, heated and applied to individual lanes. At the completion of the electrophoretic separation, the gel is silver stained to reveal residual peptides. Direct comparisons are made in the same gel using 5 micrograms of the non-hydrolyzed protein starting material.

[0020] The residual antigenicity of the hydrolysate is determined using an enzyme-linked immunosorbent assay and other nutrients such as vitamins and minerals. Typically, animal oils, vegetable oils, starch, sucrose, lactose and/or

corn syrup solids will be added to the formula to supply part or all of the above nutrients.

[0021] It is preferred that the infant formula of the invention is nutritionally complete. By the term "nutritionally complete" is meant that the composition contains adequate nutrients to sustain healthy human life for extended periods.

[0022] The amount of partial protein hydrolysate per 100 kcal of total formula is typically 1.8 g to 4.5 g; the amount of lipid source per 100 kcal of total formula is typically 3.3 g to 6 g; and the amount of carbohydrate source per 100 kcal of total formula is typically 7 g to 14 g.

[0023] The carbohydrate source in the infant formula can be any suitable carbohydrate known in the art to be suitable for use in infant formulas. Typical carbohydrate sources include sucrose, fructose, glucose, maltodextrin, lactose, corn syrup, corn syrup solids, rice syrup solids, rice starch, modified corn starch, modified tapioca starch, rice flour and soy flour.

[0024] The lipid source in the infant formula can be any lipid or fat known in the art to be suitable for use in infant formulas. Typical lipid sources include milk fat, safflower oil, egg yolk lipid, olive oil, coconut oil, palm oil, palm kernel oil, soybean oil, sunflower oil, fish oil and fractions derived thereof such as palm olein, medium chain triglycerides (MCT), and esters of fatty acids wherein the fatty acids are, for example, arachidonic acid, linoleic acid, palmitic acid, stearic acid, docosahexaeonic acid, eicosapentaenoic acid, linolenic acid, oleic acid, lauric acid, capric acid, caprylic acid, caproic acid, and the like. High oleic forms of various oils are also contemplated to be useful herein such as high oleic sunflower oil and high oleic safflower oil. Medium chain specific minerals and other vitamins will vary somewhat depending on the intended infant population.

[0025] The infant formula of the invention also typically contains emulsifiers and stabilizers such as soy lecithin and carrageenan.

[0026] The infant formula of the invention may optionally contain other substances which may have a beneficial effect such as lactoferrin, nucleotides, nucleosides and immunoglobulins.

[0027] The osmolality of the liquid infant formula of the invention (when ready to consume) is typically 100 to 500 mOsm/kg H_2O, more typically 200 to 400 mOsm/kg H_2O.

[0028] The infant formula of the invention can be sterilized, if desired, by techniques known in the art, for example, heat treatment such as autoclaving or retorting.

[0029] The infant formula of the invention can be packaged in any type of container known in the art to be used for storing nutritional products such as glass, lined paperboard, plastic and coated metal cans.

[0030] The following examples are to illustrate the invention.

EXAMPLE 1

[0031] The proteins are solubilized at 50°C at concentrations of 4-6% protein. This corresponds to 10-25% solids depending on the type of protein used. The preheat treatment constitutes holding at 75°C for 30 minutes. The mixture is cooled back to 50°C and the enzyme mixture is added. The hydrolysis can be conducted over a temperature range of 30-50°C for 2-6 hours. The hydrolysis time and temperature are interrelated, the lower temperature limit corresponding to the upper time limit and vice versa. The pH of the mixture may or may not be controlled during the hydrolysis period. To inactivate the enzyme, the hydrolysate is subjected to steam injection temperatures of 130°C for 45 seconds. This high temperature step together with the preheat treatment and enzyme hydrolysis yields a hydrolysate of reduced antigenicity. The pH is adjusted to at least 6.6, if necessary, with potassium hydroxide to prevent heat coagulation during the heat treatment.

EXAMPLE 2

[0032] A procedure as in Example 1 was performed but with a pH adjustment to 7.5 with KOH before a preheat treatment of 85°C for 10 minutes. To inactivate the enzyme, the partial hydrolysate is heated to 85°C for 10 minutes. As in Example 1, pH is adjusted to at least 6.6 before the latter step.

[0033] Different process variables are shown in the following table to illustrate preparation of hydrolysates according to Examples 1 and 2. In all cases the weight ratio of whey protein:casein was 80:20.

VARIABLE		PROCESS EXAMPLE	% D.H.	% REDUCTION IN ANTIGENICITY (HYDROLYSATE)
Trypsin to chymotrypsin ratio	1.3	2	6.77	92.5
	5.3	2	6.50	94.3
	8.8	2	7.24	93.7

(continued)

VARIABLE		PROCESS EXAMPLE	% D.H.	% REDUCTION IN ANTIGENICITY (HYDROLYSATE)
Enzyme concentration (% of Protein)	0.8	2	5.50	92.7
	0.6	2	4.88	94.3
	0.4	2	4.72	91.3
Hydrolysis time (Hrs.)	2	1	4.53	90.6
	2	2	4.55	91.6
	3	1	4.57	92.4
	3	2	5.48	93.9
	4	1	5.35	92.4
	4	2	6.50	94.3
	6	2	6.73	94.6
Hydrolysis temperature (°C)	30	2	4.53	90.6
	40	2	5.71	91.6
	50	2	5.76	94.6

EXAMPLE 3

[0034] For a liquid infant formula, to the partial hydrolysate from Example 1, is added lactose and minerals dissolved beforehand. The mixture is heated to 70°C in a plate type heat exchanger. This is followed by the introduction of fats which consists of palm olein, sunflower oil, coconut oil, soy oil, lecithin, mono-and diglycerides and fat soluble vitamins. The oils are melted before addition to the mixture. After preheating to about 75°C, the mixture is heated to 140°C for 45 seconds by direct injection of steam and cooled to 70°C by a plate cooler. This is then followed by homogenization in two stages, first at 175 bar and then at 35 bar. The mixture is then cooled to 5-7°C with a plate cooler and stored in an intermediate storage tank where water soluble vitamins are added. The bulk product from this process has a heat coagulation time of more than 30 minutes. The product is sterilized in conventional retort systems.

[0035] The liquid has the following composition:

Peptides	1.65 %
Fat	3.60 %
Carbohydrates	6.52 %
Minerals	0.48 %
Vitamins	Trace

EXAMPLE 4

[0036] For another liquid infant formula, a procedure as in Example 3 and partial hydrolysate from Example 2, except that direct steam injection temperature is 121°C and the liquid is sterilized at 145°C for 4.97 seconds and aseptically packed in containers. Composition is the same as in Example 3.

EXAMPLE 5

[0037] For an infant formula powder, a procedure as in Example 3 with a partial hydrolysate from either Example 1 or 2, except that the product is not subjected to steam injection temperatures before a 2-stage homogenization step of 125 bar and 50 bar. The powder base can be evaporated to 50% solids before spray drying.

[0038] The powder has the following composition:

Peptides	13.1 %
Fat	28.6 %
Carbohydrates	51.8 %
Minerals	3.8 %
Vitamins	0.2 %
Moisture	2.5 %

[0039] Infant formulas produced according to processes in Examples 1 to 5 demonstrate reduced antigenicity as shown in the following table.

PRODUCT FORM (Whey protein:casein weight ratio. Protein Source)	PROCESS EXAMPLE	% REDUCTION IN ANTIGENICITY
Liquid (80:20. UF* whey, NFDM**)	Examples 1, 4	92.4
Liquid (80:20. UF whey, NFDM)	Examples 2, 4	94.3
Liquid (80:20. UF whey, NFDM)	Examples 1, 3	92.1
Liquid (80:20. UF whey, casein)	Examples 1, 4	81.4
Liquid (60:40. UF whey, NFDM)	Examples 1, 4	87.8
Liquid (60:40. ED*** whey, NFDM)	Examples 1, 4	94.6
Powder (80:20. UF whey, NFDM)	Examples 2, 5	92.3

* UF = ulrafiltered

** NFDM = non-fat dried milk

*** ED = electrodialyzed

EXAMPLE 6

[0040] A 40-member consumer panel evaluates and compares a formula of the invention substantially as described in Example 4 to a commercial formula (Good Start™ Iron Fortified Infant Formula, available from Carnation Company, Glendale, CA, USA) having similar ingredients except that the protein component is partially hydrolyzed 100% whey protein. Samples are served at room temperature and evaluated by the panel. Specific and sensory attributes of the infant formula such as bitterness, aftertaste, mouthfeel, as well as overall flavor score, are compared using a 9-point hedonic scale (9=like extremely, 1=dislike extremely). The results are in the following table.

	Formula of the Invention	Good Start
Appearance	7.3	6.7 *
Flavor	4.0	3.3 *
Flavor Strength	4.5	4.0
Sweetness	4.8	4.1 *
Milky	5.4	5.1
Buttery	5.0	4.5
Meaty	4.4	4.1
Bitter	3.6	3.6
Aftertaste	3.0	3.7
Overall	4.2	3.6 *
Preference	68%	32% *
Comments	Bitter 8% Poor Color 8%	Bitter 18%

*Indicate significant difference between samples at 95% confidence level.

[0041] The formula of the invention is significantly preferred over Good Start. Panelists' comments indicated that the bitter nature of Good Start is the primary reason for the preference of the formula of the invention over Good Start.

The invention has been described in detail with particular reference to preferred embodiments thereof.

Claims

1. A partial hydrolysate of a protein mixture wherein said protein mixture comprises whey protein and casein and wherein the hydrolysate has a degree of hydrolysis between 4 and 10%.

2. The partial hydrolysate of Claim 1 having a reduction in antigenicity of at least about 80% relative to the corresponding non-hydrolyzed protein mixture as measured by ELISA.

3. The partial hydrolysate of Claim 1 having a reduction in antigenicity of at least about 85% relative to the corresponding non-hydrolyzed protein mixture as measured by ELISA.

4. The partial hydrolysate of Claim 1 having a reduction in antigenicity of at least about 90% relative to the corresponding non-hydrolyzed protein mixture as measured by ELISA.

5. The partial hydrolysate of Claim 1 having a reduction in antigenicity of at least about 95% relative to the corresponding non-hydrolyzed protein mixture as measured by ELISA.

6. The partial hydrolysate of any of Claims 1 - 5 comprising 60 to 80 weight % whey protein and 40 to 20 weight % casein protein.

7. The partial hydrolysate of any of Claims 1 - 5 comprising 40 to 60 weight % whey protein and 60 to 40 weight % casein protein.

8. The partial hydrolysate of any of Claims 1 - 7 having an average molecular weight of 2,000, a maximum molecular weight of 19,000 and comprising of peptides spread over the following distribution, as a function of their molar mass:

Molar Mass (g per mole)	% Molecular Weight Distribution
MM > 5000	8.2
5000 > MM > 3000	14.5
3000 > MM > 2000	15.8
2000 > MM > 1000	26.2
1000 > MM > 500	17.8
MM < 500	17.5

9. A process for preparing a partial hydrolysate of a protein mixture comprising contacting a mixture of whey protein and casein with an enzyme mixture comprising at least 1800 USP Trypsin Units/mg and at least 350 USP Chymotrypsin Units/mg in an aqueous suspension under conditions to result in a degree of hydrolysis between 4 and 10%, wherein the ratio in USP units of Trypsin to Chymotrypsin is 1.3 to 18.

10. The process of Claim 9 wherein the ratio in USP units of Trypsin to Chymotrypsin is 1.5 to 10.

11. The process of any of Claims 9 or 10 wherein the amount of enzyme mixture is between 0.4 and 1.2%, based on the weight of the protein in the mixture.

12. The process of any of Claims 9 - 11 performed at a temperature of 30°C to 50°C, a pH of 6.5 to 8.0, and for 2 to 6 hours.

13. The process of any of Claim 9 - 12 including a pretreatment step comprising of heating the protein mixture at 75°C to 85°C for 10 minutes to 30 minutes.

14. The process of any of Claims 9 - 13 followed by the additional step of inactivating the enzyme mixture.

15. The process of Claim 14 wherein the enzyme mixture is inactivated by heating to about 85°C for about 10 minutes

or 130°C for about 45 seconds.

16. The process of Claim 15 followed by the additional step of removing water by evaporation or spray drying.

17. An infant formula comprising a partial hydrolysate of a protein mixture wherein said protein mixture comprises whey protein and casein and wherein the hydrolysate has a degree of hydrolysis between 4 and 10%.

18. The infant formula of Claim 17 which is aseptically sterilized.

19. The infant formula of Claim 17 which is retort sterilized.

20. The infant formula of any of Claims 17 - 19 that is in a powder form.

21. The infant formula of any of Claims 17 - 20 having a reduction in antigenicity of at least about 80% relative to the corresponding non-hydrolyzed protein mixture, as measured by ELISA.

22. The infant formula of any of Claims 17 - 21 wherein the taste is improved relative to a corresponding formula made with partially hydrolyzed whey protein.

Patentansprüche

1. Teilhydrolysat eines Proteingemisches, wobei das Proteingemisch Molkeprotein und Kasein beinhaltet und das Hydrolysat einen Hydrolysegrad von 4 bis 10 % besitzt.

2. Teilhydrolysat nach Anspruch 1 mit einer durch ELISA bestimmten wenigstens etwa 80 %-igen Antigenitätsverminderung, bezogen auf das entsprechende nichthydrolysierte Proteingemisch.

3. Teilhydrolysat nach Anspruch 1 mit einer durch ELISA bestimmten wenigstens etwa 85 %-igen Antigenitätsverminderung, bezogen auf das entsprechende nichthydrolysierte Proteingemisch.

4. Teilhydrolysat nach Anspruch 1 mit einer durch ELISA bestimmten wenigstens etwa 90 %-igen Antigenitätsverminderung, bezogen auf das entsprechende nichthydrolysierte Proteingemisch.

5. Teilhydrolysat nach Anspruch 1 mit einer durch ELISA bestimmten wenigstens etwa 95 %-igen Antigenitätsverminderung, bezogen auf das entsprechende nichthydrolysierte Proteingemisch.

6. Teilhydrolysat nach einem der Ansprüche 1 bis 5, umfassend 60 bis 80 Gew.-% Molkeprotein und 40 bis 20 Gew.-% Kaseinprotein.

7. Teilhydrolysat nach einem der Ansprüche 1 bis 5, umfassen 40 bis 60 Gew.-% Molkeprotein und 60 bis 40 Gew.-% Kaseinprotein.

8. Teilhydrolysat nach einem der Ansprüche 1 bis 7 mit einem mittleren Molekulargewicht von 2000, einem maximalen Molekulargewicht von 19000, und das als Funktion ihrer Molmasse folgendermaßen verteilte Peptide beinhaltet:

Molargewicht (g pro Mol)	% Molekulargewichtsverteilung
MG > 5000	8,2
5000 > MG > 3000	14,5
3000 > MG > 2000	15,8
2000 > MG > 1000	26,2
1000 > MG > 500	17,8
MG < 500	17,5

9. Verfahren zur Herstellung eines Teilhydrolysats eines Proteingemisches, wobei man auf ein Gemisch aus Molkeprotein und Kasein ein wenigstens 1800 USP Trypsin-Einheiten/mg und wenigstens 350 USP Chymotrypsin-Ein-

heiten/mg umfassenden Enzymgemisch in einer wässrigen Suspension unter Bedingungen einwirken lässt, die zu einem Hydrolysegrad von 4 % bis 10 % führen, wobei das Verhältnis von Trypsin zu Chymotrypsin in USP-Einheiten 1,3 bis 18 beträgt.

10. Verfahren nach Anspruch 9, wobei das Verhältnis von Trypsin zu Chymotrypsin in USP-Einheiten von 1,5 bis 10 beträgt.

11. Verfahren nach einem der Ansprüche 9 oder 10, wobei die Menge des Enzymgemisches 0,4 % bis 1,2 % beträgt, bezogen auf das Gewicht des Proteins im Gemisch.

12. Verfahren nach einem der Ansprüche 9 bis 11, das bei einer Temperatur von 30 °C bis 50 °C, und einem pH von 6,5 bis 8,0 zwei bis sechs Stunden durchgeführt wird.

13. Verfahren nach einem der Ansprüche 9 bis 12 mit einem Vorbehandlungsschritt, mit dem man das Proteingemisch 10 Minuten bis 30 Minuten bei 75 °C bis 85 °C erwärmt.

14. Verfahren nach einem der Ansprüche 9 bis 13, wobei in einem anschließenden Schritt das Enzymgemisch inaktiviert wird.

15. Verfahren nach Anspruch 14, wobei das Enzymgemisch durch etwa 10-minütiges Erwärmen auf 85 °C oder 45-sekündiges Erwärmen auf 130 °C inaktiviert wird.

16. Verfahren nach Anspruch 15, wobei man anschließend in einem zusätzlichen Schritt Wasser durch Verdampfen oder Sprühtrocknung entfernt.

17. Kinderfertignahrung, umfassend ein Teilhydrolysat eines Proteingemisches, wobei das Proteingemisch Molkeprotein und Kasein beinhaltet und das Hydrolysat einen Hydrolysegrad von 4% bis 10% besitzt.

18. Kinderfertignahrung nach Anspruch 17, die aseptisch sterilisiert ist.

19. Kinderfertignahrung nach Anspruch 17, die retortensterilisiert ist.

20. Kinderfertignahrung nach einem der Ansprüche 17 bis 19 in Pulverform.

21. Kinderfertignahrung nach einem der Ansprüche 17 bis 20 mit einer durch ELISA bestimmten wenigstens etwa 80 %-igen Antigenitätsverminderung, bezogen auf das entsprechende nichthydrolysierte Proteingemisch.

22. Kinderfertignahrung nach einem der Ansprüche 17 bis 21, deren Geschmack im Vergleich zu einer entsprechenden mit teilhydrolysiertem Molkeprotein zubereiteten Fertignahrung verbessert ist.

Revendications

1. Hydrolysat partiel d'un mélange de protéines où ledit mélange de protéines comprend de la protéine de petit lait et de la caséine et où l'hydrolysat a un degré d'hydrolyse entre 4 et 10%.

2. Hydrolysat partiel de la revendication 1 ayant une réduction d'antigénicité d'au moins environ 80% relativement au mélange de protéines non hydrolysé correspondant en mesurant par ELISA.

3. Hydrolysat partiel de la revendication 1 ayant une réduction d'antigénicité d'au moins environ 85% relativement au mélange de protéines non hydrolysé correspondant en mesurant par ELISA.

4. Hydrolysat partiel de la revendication 1 ayant une réduction d'antigénicité d'au moins environ 90% relativement au mélange de protéines non hydrolysé correspondant en mesurant par ELISA.

5. Hydrolysat partiel de la revendication 1 ayant une réduction d'antigénicité d'au moins environ 95% relativement au mélange de protéines non hydrolysé correspondant en mesurant par ELISA.

6. Hydrolysat partiel selon l'une quelconque des revendications 1-5 comprenant 60 à 80 pour cent en poids de protéine de petit lait et 40 à 20 pour cent en poids de protéine de caséine.

7. Hydrolysat partiel selon l'une quelconque des revendications 1 - 5 comprenant 40 à 60 % en poids de protéine de petit lait et 60 à 40 % en poids de protéine de caséine.

8. Hydrolysat partiel selon l'une quelconque des revendications 1 - 7 ayant un poids moléculaire moyen de 2000, un poids moléculaire maximum de 19000 et comprenant des peptides répartis sur la distribution suivante, en fonction de leur masse molaire:

Masse Molaire (g par mole)	%Distribution Poids Moléculaire
MM > 5000	8,2
5000 > MM > 3000	14,2
3000 > MM > 2000	15,8
2000 > MM > 1000	26,2
1000 > MM > 500	17,8
MM < 500	17,5

9. Procédé de préparation d'un hydrolysat partiel d'un mélange de protéines comprenant la mise en contact d'un mélange de protéine de petit lait et de caséine avec un mélange d'enzymes comprenant au moins 1800 Unités USP de Trypsine/mg et au moins 350 Unités USP de Chymotrypsine/mg dans une suspension aqueuse dans des conditions devant avoir pour résultat un degré d'hydrolyse entre 4 et 10%, où le rapport en unités USP de la Trypsine à la Chymotrypsine est de 1,3 à 18.

10. Procédé de la revendication 9 où le rapport en unités USP de la Trypsine à la Chymotrypsine est de 1,5 à 10.

11. Procédé selon l'une quelconque des revendications 9 ou 10 où la quantité du mélange d'enzymes est entre 0,4 et 1,2%, en se basant sur la poids de la protéine dans le mélange.

12. Procédé selon l'une quelconque des revendications 9 - 11 accompli à une température de 30°C à 50°C, un pH de 6,5 à 8,0, et pendant 2 à 6 heures.

13. Procédé selon l'une quelconque des revendications 9 - 12, comprenant une étape de prétraitement comprenant le chauffage du mélange de protéines à 75°C jusqu'à 85°C pendant 10 minutes à 30 minutes.

14. Procédé selon l'une quelconque des revendications 9 - 13 suivi de l'étape additionnelle d'inactivation du mélange d'enzymes.

15. Procédé de la revendication 14 où le mélange d'enzymes est inactivé par chauffage aux environs de 85°C pendant environ 10 minutes ou 130°C pendant environ 45 secondes.

16. Procédé de la revendication 15 suivi de l'étape additionnelle d'enlèvement de l'eau par évaporation ou séchage par pulvérisation.

17. Formule pour nourisson comprenant un hydrolysat partiel d'un mélange de protéines où ledit mélange de protéines comprend de la protéine de petit lait et de la caséine et où l'hydrolysat a un degré d'hydrolyse entre 4 et 10%.

18. Formule pour nourisson de la revendication 17 qui est aseptiquement stérilisée.

19. Formule pour nourisson de la revendication 17 qui est stérilisée à la cornue.

20. Formule pour nourisson de l'une quelconque des revendications 17 - 19 qui est en forme de poudre.

21. Formule pour nourisson de l'une quelconque des revendications 17 - 20 ayant une réduction d'antigénicité d'au moins environ 80% relativement au mélange de protéines non hydrolysé correspondant en mesurant par ELISA.

22. Formule pour nourisson selon l'une quelconque des revendications 17 - 21 où le goût est amélioré relativement à une formule correspondante faite avec de la protéine de petit lait partiellement hydrolysée.